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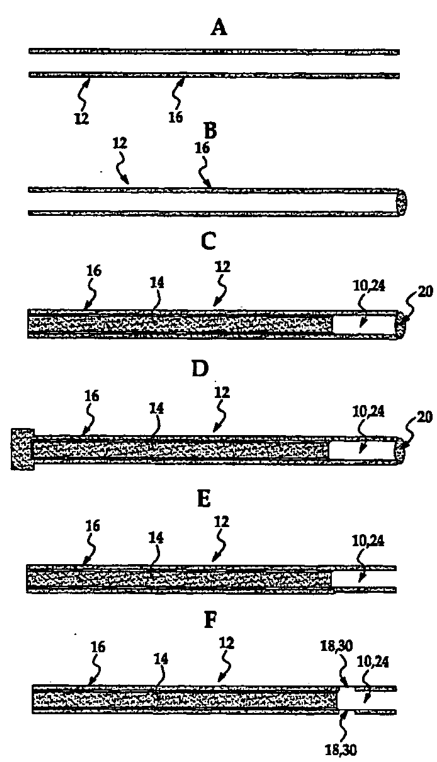
(54) Title: SOL-GEL MONOLITHIC COLUMN WITH OPTICAL WINDOW AND METHOD OF MAKING



(57) Abstract: A method of preparing a sol-gel monolithic column includes the step of forming a separation bed (14) from a sol-gel solution in a single process step. This column has improved characteristics for CEC based on its incorporated surface charge and ease of operation due to a lack of or need for end frits. Also, a second type of column includes an optical window (30) for on-column detection.

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SOL-GEL MONOLITHIC COLUMN WITH OPTICAL WINDOW AND METHOD OF MAKING

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention relates to columns and methods of making columns for separation techniques and apparatus. More specifically, the present invention provides a separation bed and method of making the same for use in various electromigration and non-electromigration separation columns, such as high-performance liquid chromatography, gas chromatography, capillary electrophoresis, capillary electrochromatography, and supercritical fluid chromatography.

2. DESCRIPTION OF RELATED ART

Capillary electrochromatography or CEC is a fairly novel electrokinetic separation technique representing a hybrid of high-performance liquid chromatography or HPLC and capillary electrophoresis, known as CE. In CEC, the electroosmotic flow, or EOF is used to drive the mobile phase through the capillary, using typical HPLC mobile and stationary phases that provide the essential chromatographic interactions. Because of the flat plug-like profile of the electroosmotic flow, CEC offers greatly enhanced separation efficiencies relative to HPLC. Unlike CE, CEC is not restricted to charged solutes. Thus, the potential for CEC, as a separation technique, is much wider.

Capillary electrochromatography is a rapidly growing area in analytical separations. A great deal of research effort is currently being devoted to materialize the great analytical potential that this new hybrid technique has to offer. In order for CEC to achieve success as an independent chromatographic separation technique significant advancements are needed in the area of column technology. This is explained by the fact that in CEC, the column not only serves as the separation chamber, but also as the pumping device to drive the mobile phase through the system. This makes the column the "heart" of the CEC system both in the functional and literal sense of the word.

Two major types of columns are used in current CEC practice. These are packed and open tubular types. Packed columns comprise the predominant class of CEC columns. Most often the packed capillaries contain 1.5 – 5 μm, non-polar, octadecylated or ODS particles. The ODS particles possess both the chemically bonded octadecyl stationary phase, providing the essential chromatographic interactions, and the silanol moieties, responsible for the generation of electroosmotic flow to drive the mobile phase and the solutes through the packed capillary. The commercial availability of the ODS-bonded particles and the previously established liquid chromatography or LC separation protocols are two advantages attracting many researchers to use these packed capillaries in CEC. However, the most significant advantage of packed columns in CEC is the possibility of using small micrometer and nanometer size particles. High separation efficiency during fast analysis is achieved in packed CEC columns without requiring ultra-high pressures, as in HPLC to drive the mobile phase through the columns packed with the small particles.

The greatest challenge is the preparation of a uniform packing bed using the small particles. Researchers currently use a variety of packing procedures ranging from slurry packing, electrokinetic, centripetal, and supercritical fluid packing methods. These all involve a plurality of steps to effect the packing process and even with close monitoring do not give as uniform a bed as desired for many applications.

Furthermore, a great degree of difficulty still remains associated with the ability to pack long, narrow bore capillaries. In addition, most packed capillaries require end frits of a different material to retain the packing particles within the packed capillary bed. Creation of those frits remains to be a problem in column preparation as these frits must be rigid enough to retain the packing particles under a wide range of column packing, rinsing and operating conditions. Yet these frits must also possess a highly porous structure to permit a uniform mobile phase flow through the entire cross-section of the column. A further problem arises in that the presence of the frit material makes the packing in the column non-homogeneous due to the presence of a different material and this can cause problems with the separation characteristics of the final column.

Monolithic column technology can effectively overcome both of the difficulties associated with conventional packed capillary column technology. In the monolithic

approach, a continuous separation bed is created inside the capillary tube using a solution, which undergoes both chemical and physical changes in the capillary environment to produce the separation bed. In addition, the choice of appropriate chemistry allows the porous bed to chemically bond to the inner walls of the capillary by a condensation reaction and the resulting packed tube is also homogeneous in nature.

The use of monolithic columns has been reported in gas and liquid chromatography and is also currently being used in CEC to alleviate the extensive labor involved with packed column fabrication. Moreover, the greatest inherent advantage of the monolithic capillary columns is the elimination of the need for the end frits. The elimination of these end frits allows the entire column to remain homogeneous, rather than exhibiting different properties by the packing particles and the end frits. It has also been demonstrated that the end frits reduce the column's separation efficiency and are responsible for bubble formation during the analysis.

Although much simpler than particle packed capillaries, monolithic columns derived by organic polymerization also possess certain limitations. One critical drawback associated with this type of monolithic capillary is the tendency of the polymer network to swell during exposure to certain organic solvents, which are contained in the running mobile phase. This swelling may result in reductions in the permeability of the monolith as a result of alterations in the porosity of the monolith. Such structural change ultimately leads to changes in the column performance during the course of its use.

Unlike the monolithic separation beds from organic polymers, columns containing a porous silica-based monolithic matrix prepared through sol-gel chemistry do not suffer from the swelling phenomena thus offering a versatile and promising alternative to organic packed capillaries. In addition, monolithic columns, since they are prepared without end frits can produce a homogeneous separation column, which is highly desirable for a wide variety of separation techniques.

Pretorius was one of the first influential pioneers of CEC who, in 1974, demonstrated the advantages of electroosmosis as a pumping mechanism for chromatographic separations. Jorgenson and Lukacs published CEC analyses of 9-methylanthracene and perylene on an ODS-packed capillary column. Meanwhile, a 1987 report by Tsuda demonstrated the possibility of achieving CEC separations by the

simultaneous use of both electroosmotic and pressure-driven flows in the separation column. Yet Knox and Grant made another significant contribution to the development of this technique. Following this publication, the term "electrochromatography" became generally accepted and numerous researchers refocused their attention to CEC.

As described earlier, two types of monolithic columns have been developed: (1) organic polymer-based and (2) bonded silica-based. In the first approach, fabrication of a monolithic capillary column is accomplished by polymerization reaction of organic monomeric precursor(s). Hileman et al used Carbowax coated open pore polyurethane monolithic capillaries for the separations of several classes of analytes including aromatic hydrocarbons, aliphatic alcohols and metal chelates through gas chromatography. Hjerten et al prepared monolithic capillaries with compressed polyacrylamide gels for separation of proteins using HPLC and of low molecular mass compounds and basic proteins using CEC. Frechet and coworkers reported a series of publications on the use of methacrylate monomers for the preparation of HPLC and CEC monolithic capillaries through copolymerization. Palm and Novotny prepared CEC monoliths using mixtures of polyacrylamide/polyethylene glycol, derived with either C_4 or C_{12} ligands, which were used to separate alkyl phenones and peptides. Additionally, Fujimoto et al reported the usage of cross-linked polyacrylamides for the separation of small dansylated amino acids and neutral steroids on monolithic CEC capillaries.

An alternative to a column with an organic polymer-based stationary phase column is one with a bonded silica stationary phase prepared by sol-gel chemistry. Cortes and coworkers prepared porous beds by polymerizing potassium silicate solutions *in situ*. The columns containing the porous beds were then packed with 5 μm Spherisorb ODS particles for use in LC. Fields used solutions of potassium silicate and formamide to create a porous bed that was further reacted with dimethyloctadecylchlorosilane, and achieved plate heights of 65 μm in LC.

Tanaka and coworkers used the sol-gel technique for the development of an octadecylsilylated, porous monolithic column for use in LC. In this study, poly(ethylene oxide), PEO, was incorporated into a mixture of tetramethoxysilane (TMOS) and acetic acid to develop porous silica rods, followed by an in-column octadecylsilylation reaction.

Following washings, and drying at 50°C for three days, the silica rods were then treated for two hours at 600°C.

Dulay et al used sol-gel technology for the preparation of monolithic columns loaded with 3 μm ODS particles. The sol-gel solution served as a retaining matrix immobilizing and shielding the ODS stationary phase particles. Sol-gel capillary columns containing the ODS embedded particles yielded CEC separation efficiencies on the order of 80,000 plates/m (16,000 plates/column) for a test mixture of six uncharged polyaromatic hydrocarbons or PAHs.

Lee and coworkers also used sol-gel chemistry to glue 7 μm ODS particles thereby creating a continuous large-pore CEC column. The sol-gel technology in this approach was used to create a bridge between adjacent particles, as well as the capillary wall and particles in its vicinity, thereby eliminating the need for retaining end-frits, thus result being efficient separations of small organic and aromatic amine compounds on such "sol-gel-glued" monolithic columns.

SUMMARY OF THE INVENTION

It is, therefore, an object of the invention to create a monolithic column from a sol-gel process.

It is also an object of the invention to prepare a sol-gel column having a porous, separation bed without the use of particles being incorporated into the bed.

It is a further object of the invention to create a sol-gel column in a single-step process that obviates the need for a plurality of processing steps.

It is another object of the invention to produce a monolithic sol-gel column, which is chemically bonded to the capillary wall.

It is a further object of the invention to produce a monolithic sol-gel column that does not require high temperature processing steps.

It is another object of the invention to produce a monolithic sol-gel column having an optical window useful for on-column detection studies of the analytes separated by the separation column, using various spectral techniques.

BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention are readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIGURE 1 is a scanning electron micrograph of a sol-gel monolithic column, cross-sectional view, magnified 1,800 times;

FIGURE 2 is a scanning electron micrograph of a sol-gel monolithic column, longitudinal view, magnified 7,000 times;

FIGURE 3 is a scanning electron micrograph of a sol-gel monolithic column, longitudinal view, magnified 15,000 times;

FIGURE 4 is a graph of the effect of the change of electroosmotic mobility with an increase in percentage of acetonitrile and Tris-HCl in a mobile phase;

FIGURE 5 represents the CEC analysis of a mixture of PAHs on a 50 cm x 50 μm ODS sol-gel monolithic column;

FIGURE 6 shows plate height versus flow rate within a sol-gel mediated ODS monolithic capillary;

FIGURE 7 is a separation analysis of a mixture of benzene derivatives on a sol-gel mediated ODS monolithic column;

FIGURE 8 is a CEC separation of a mixture of aldehydes and ketones on a sol-gel mediated ODS monolithic column; and

FIGURE 9 is a schematic showing the various steps in preparing a monolithic separation column having an additional optical window in the structure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, monolithic sol-gel columns are prepared by an *in situ* creation of chromatographic stationary phases with surface-bonded ligands. Unlike conventional techniques, various column preparation processes, such as deactivation, coating/packing, stationary-phase immobilization and end frit making, are carried out in one single step, thus reducing the time and labor associated with column fabrication. In

addition, the process produces a column that is homogenous since there are no particles included in the sol-gel and the sol-gel monolithic bed actually forms bonds with the fused silica capillary surface, making a unitary structure across the diameter of the tube.

In order to achieve the desired sol-gels of the instant invention, certain reagents in a reagent system were preferred for the fabrication of the gels for the monolithic columns of the present invention. The reagent system included two sol-gel precursors, a deactivation reagent, one or more solvents and a catalyst. For the purposes of this invention, one of the sol-gel precursors contains a chromatographically active moiety selected from the group consisting of octadecyl, octyl, cyanopropyl, diol, biphenyl, phenyl, cyclodextrins, crown ethers and other moieties. Representative precursors include, but are not limited to: Tetramethoxysilane, 3-(N-styrlmethyl-2-aminoethylamino)-propyltrimethoxysilane hydrochloride, *N*-tetradecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride, N(3-trimethoxysilylpropyl)-N-methyl-N,N-diallylammonium chloride, N-trimethoxysilylpropyltri-N-butylammonium bromide, N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride, Trimethoxysilylpropylthiouronium chloride, 3-[2-N-benzyaminoethylaminopropyl]trimethoxysilane hydrochloride, 1,4-Bis(hydroxydimethylsilyl)benzene, Bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane, 1,4-bis(trimethoxysilylethyl)benzene, 2-Cyanoethyltrimethoxysilane, 2-Cyanoethyltriethoxysilane, (Cyanomethylphenethyl)trimethoxysilane, (Cyanomethylphenethyl)triethoxysilane, 3-Cyanopropyldimethylmethoxysilane, 3-Cyanopropyltriethoxysilane, 3-Cyanopropyltrimethoxysilane, *n*-Octadecyltrimethoxysilane, *n*-Octadecyldimethylmethoxysilane, Methyl-*n*-Octadecyldiethoxysilane, Methyl-*n*-Octadecyldimethoxysilane, *n*-Octadecyltriethoxysilane, *n*-Dodecyltriethoxysilane, *n*-Dodecyltrimethoxysilane, *n*-Octyltriethyoxysilane, *n*-Octyltrimethoxysilane, *n*-Ocyldiisobutylmethoxysilane, *n*-Octylmethyldimethoxysilane, *n*-Hexyltriethoxysilane, *n*-isobutyltriethoxysilane, *n*-Propyltrimethoxysilane, Phenethyltrimethoxysilane, N-Phenylaminopropyltrimethoxysilane, Styrylethyltrimethoxysilane, 3-(2,2,6,6-tetramethylpiperidine-4-oxy)-propyltriethoxysilane, N-(3-triethoxysilylpropyl)acetyl-

glycinamide, (3,3,3-trifluoropropyl)trimethoxysilane, and (3,3,3-trifluoropropyl)methyldimethoxysilane.

A second sol-gel precursor, *N*-Octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride, was found to be critical since it possessed an octadecyl moiety that allowed for chromatographic interactions of analytes with the monolithic stationary phase. Additionally, this reagent served to yield a positively charged surface thereby providing the relatively high electroosmotic flow necessary in capillary electrochromatography. However, it is considered within the scope to use any other reagent as known to one of ordinary skill in the art that would contain the octadecyl moiety for the purposes already set forth.

The deactivation reagent, Phenyldimethylsilane, and the catalyst, Trifluoroacetic acid, were selected for the preparation of the columns of the instant invention, however, any know deactivation reagent and/or catalyst as known to those of ordinary skill in the art may be used.

The sol-gel solutions were prepared by mixing 100 μL of Tetramethyloxysilane (TMOS) with 100 μL of C_{18}-TMS (N-Octyldecyl-dimethyl[3-(trimethoxysilyl)propyl]ammonium chloride), 10 μL of PheDMS (Phenyldimethylsilane), 100 μL of 99% Trifluoroacetic acid (TFA) (containing 10% water), and 100 μL of 90% TFA (containing 10% water) in a micro vial. This mixture was thoroughly vortexed for 5 minutes, and the precipitate was then separated from the sol-gel solution through centrifugation at 13,000 rpm for 5minutes. The supernatant was decanted into another micro vial and used for the creation of the monolithic separation bed.

A standard fused silica capillary tube, as known to those of skill in the art, was selected to be filled with the sol-gel solution. The tube used here was externally coated with polyimide, however it is within the scope of the invention to use a tube coated with any polymer or other coating such as metal, as known in the art; the external coating serving as a structural integrity device for the tube.

Prior to filling with the sol-gel solution, the inner surface of the capillary was first treated with deionized water. For this, an approximately 5 meter long section of 50 μm internal diameter fused silica capillary was rinsed with deionized water for approximately 15 minutes under a helium pressure of 200 psi. The capillary was then emptied by

expelling the water from within by using the same helium pressure. Both ends of the capillary tube were then fused using an oxyacetylene torch, and the capillary was placed in a GC oven for thermal conditioning by raising the temperature at 0.5°C/min from 40°C to a final temperature of 250°C with a hold time of 60 minutes at 250°C. The column was then removed from the GC oven, and the ends were opened, followed by purging of the column with helium under 200 psi pressure for an additional 30 minutes.

Next, a desired length, for example 60 cm, of the hydrothermally pretreated fused-silica capillary was taken and installed into the capillary filling chamber, containing a polyethylene microcentrifuge vial with the desired sol-gel solution. It is, of course, within the scope of the invention to use any desired length as desired by one of skill in the art. Using 100 psi helium pressure, the sol solution was pushed into the column. The column, containing the sol solution, was then allowed to remain installed in the pressurized capillary chamber and left undisturbed for approximately four hours until gelation of the sol solution was visually apparent. Following this, the pressure was slowly released and the column was removed from the capillary filling/purging chamber. It was then affixed perpendicular to the bench top. A 60-s epoxy seal was then applied to the ends of the capillary to ensure adequate sealing prior to its thermal conditioning. Next, a very slow thermal conditioning program was used. An example of this thermal conditioning consists of a programmed temperature heating at 0.2°C/min from 35°C (1minute hold time) to a final temperature of 150°C, where the column has held for 120 minutes. Following heating, the ends were cut open and the monolithic capillary was then installed into a Bio-Rad CE system for subsequent rinsing at 100 psi. It is, of course, contemplated that any CE system as known to those of skill in the art may also be used. The monolithic column was initially rinsed with 100% HPLC grade acetonitrile, followed by a 50:50 acetonitrile/deionized water solution for periods of 5 minutes each, and finally the desired running mobile phase for 15 minutes prior to conducting column evaluation and/or analysis.

Visualization of the monolithic microstructure within the capillary tube was accomplished through the use of a scanning electron microscope. All scanning electron micrographic images were acquired from sections of the monolithic column initially cut into equal lengths, those being approximately 2.5 mm, and positioned perpendicularly

within a retractable aluminum stage using a double-sided tape. These samples were then used to obtain cross-sectional views of the monolithic CEC columns. Longitudinal sections were acquired by dissecting approximately 1.0 cm sections of capillary at approximately 45°, thus yielding a capillary segment revealing a protruding portion of the monolithic matrix without the top portion of the fused silica present. These sections were then mounted parallel on an aluminum stage with the aid of double-sided carbon tape. Both stages, with all mounted capillary segments, were then consecutively placed into a Balzers SCD 050 sputter coating chamber and coated with a gold/palladium alloy at 40 mA for 60 seconds to avert subsequent charging.

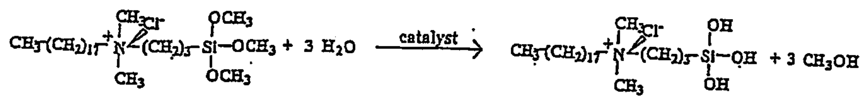
FIGURE 1 represents a SEM cross-sectional view of the sol-gel monolithic column at a magnification of 1800x. Observations at this magnification reveal that the entire cross-section of the capillary contains the monolithic matrix. FIGURE 2, a longitudinal view of the monolithic capillary at 7000x magnification, reveals the porous structure of the monolithic matrix. From this view it is evident that the pore diameters are of approximately 1.5 μm. The use of higher C_{18}TMS-to-TMOS molar ratios in the sol solution provided monolithic beds with the said pore characteristics. This also allowed for enhanced permeability of the mobile phase.

For example, an increase in the C_{18}TMS-to-TMOS molar ratio of from 0.5 to 0.75 yielded flow rates of up to approximately 7.75 μL/min for the mobile phase consisting of 80% (v/v) Acrylonitrile 20% (v/v)/5 mM Tris-HCl. Dimethylsulfoxide (DMSO) was used as the neutral EOF marker to determine the linear velocity of the mobile phase and was found to be 0.97 mm/s using the mobile phase.

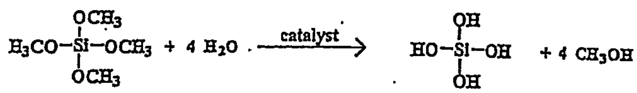
FIGURE 3, a cross-sectional view of the inner capillary at higher magnification (15000x), reveals the chemical bonding during the column preparation process that occurred due to the condensation between the sol-gel network structure and the silanol moieties on the inner capillary walls.

The scanning electron micrograph studies show that sol-gel chemistry provides a unique, yet simple mechanism for the fabrication of CEC monolithic columns. One of the key sol-gel reactions consists of the hydrolysis of the precursors. This is shown below with respect to the use of TMOS and C_{18}TMS. It is understood that this choice of reagents is for illustrative purposes only and others can be used as described before:

The Complete Hydrolysis of *N*-Octadecyldimethyl[3-(thimethyloxysilyl)propyl] ammonium chloride C_{18}-TMS (a) and tetramethoxysilane (TMOS) (b)



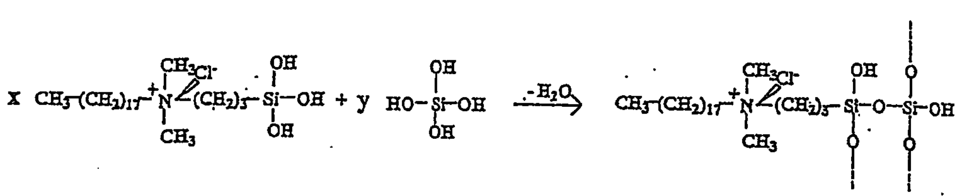
(a)



(b)

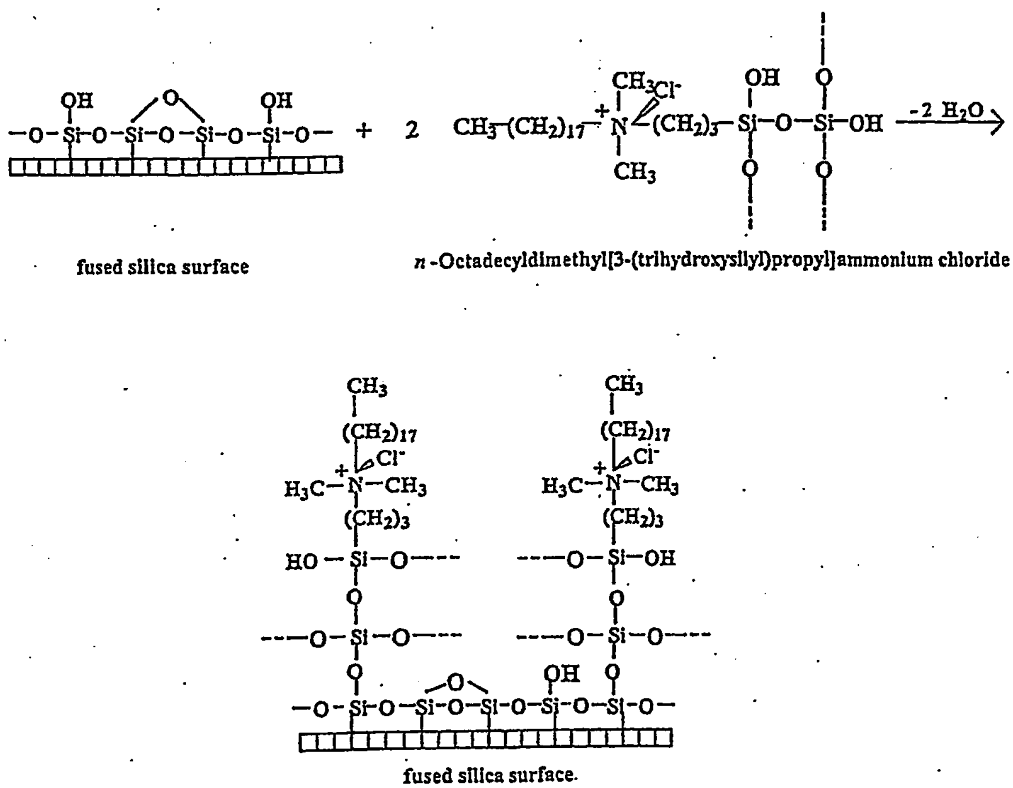
As shown above, the nucleophilic attack of water molecules on the silicon atom results in the replacement of the methoxy substituents with hydroxy moieties. As the sol-gel reactions proceed, the products of the hydrolysis can then undergo polycondensation reactions in a variety of ways: (a) between hydrolyzed products of the same original precursor, (b) between hydrolyzed products of two different original precursors, and (c) between the hydrolyzed products of either precursor with the silanol groups on the inner capillary surface. A simplified representation of a polycondensation reaction between the hydrolysis products of both precursors is depicted below:

Condensation of Tetrahydroxysilane with *N*-Octadecyldimethyl[3-(trihydroxysilyl)propyl] ammonium chloride



This growing three-dimensional polymeric network will then eventually become anchored to the inner capillary surface through chemical bonding with the silanol moieties residing along the inner fused-silica capillary surface. This is shown by the following:

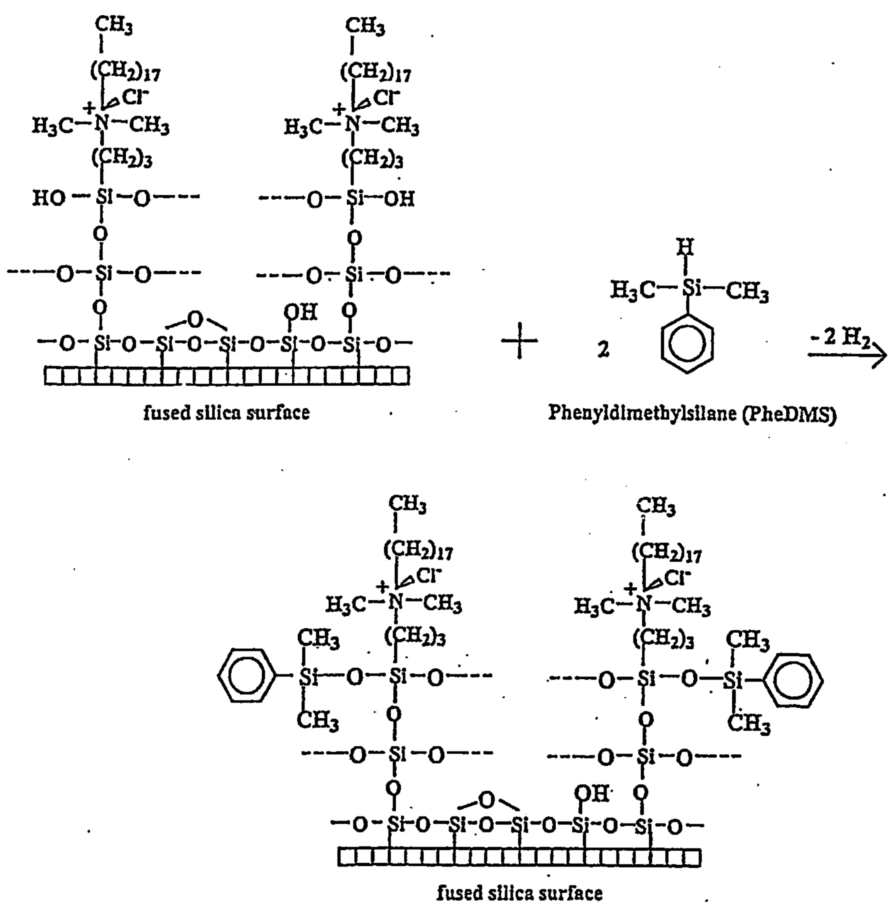
Condensation of the fused-silica surface with the growing sol-gel network containing chemically bonded residue of *N*-Octadecyldimethyl[3-(trihydroxysilyl)propyl] ammonium chloride



Finally, the incorporation of the PheDMS into the sol solution serves as a deactivating reagent for the monolithic bed. This deactivation reagent is initially added

to the sol solution. The mobile hydrogen bonded to silicon atom in the structure of this reagent is reactive toward silanol groups, especially at elevated temperatures. It can be assumed that during the sol-gel process, this reagent becomes physically incorporated in the monolithic structure but subsequently, during thermal treatment of the column, reacts with the residual silanol groups in the monolithic structure providing deactivation, as shown below:

Deactivation of the sol-gel ODS monolith with Phenyldimethylsilane.



Thus it has been shown that the sol-gel process provides a chemical anchorage of the monolithic matrix to the inner walls of the capillary (FIGURE 3). This thoroughly

illustrates a significant attribute of the sol-gel monolithic column. Another aspect is that the sol-gel monolithic bed is completely held in the column chemical bonding with the walls of the capillary, thus obviating the need for any end frits to hold the column material in place within the capillary.

Several analyses were performed using the monolithic columns of the instant invention. Each mobile phase was prepared by mixing the desired volumes of acetonitrile with a Tris-HCl background electrolyte solution. The organic solvent and the background electrolyte solution were thoroughly degassed individually via simultaneous ultrasonication and helium purging for approximately one hour prior to mixing and usage. Thorough degassing of the mobile phase was necessary to prevent subsequent bubble formation/generation during usage. This initial degassing procedure allowed for electrochromatographic experiments to be continuously performed without pressurization of the mobile phase. To achieve the desired concentration of aqueous electrolyte, a 50 mM solution was initially prepared followed by dilution to achieve the 5 mM concentration. The pH of this 5 mM solution was then measured and adjusted to approximately 2.3 by using concentrated HCl. This 5 mM Tris-HCl, having a pH of approximately 2.3 solution, in conjunction with 100% acetonitrile was individually degassed by simultaneous ultrasonication and helium purging, followed by mixing the solution in appropriate volume ratios (e.g., 75% acetonitrile/25% 5 mM Tris-HCl, etc.) to prepare the running mobile phase.

Experiments were conducted for the investigation of the electroosmotic flow (EOF) in sol-gel monolithic columns. The first measurements obtained using the monolithic ODS capillary was an evaluation of the effect of acetonitrile percentage in the running mobile phase on the electroosmotic mobility. For this, a set of mobile phases containing varying percentages of acetonitrile and 5 mM aqueous Tris-HCl was utilized. In addition, DMSO was used as the neutral electroosmotic flow marker. The results obtained from these experiments are depicted in FIGURE 4. As illustrated, the electroosmotic mobility within the ODS monolithic capillary consistently increased with the acetonitrile content in the mobile phase. Such an increase in EOF is indicative of an increase in the net positive surface charge within the monolithic columns. One possibility for this to occur is the reduction of effective negative surface charge due to an

increase in acetonitrile concentration in the mobile phase, resulting in an equivalent increase of the effective positive surface charge due to the quaternary ammonium groups. This is possible because the negative charge on the monolith/capillary is reduced due to the interaction of acetonitrile with the negative-charge generating surface groups such as the silanols.

In CEC, a consistent EOF is essential to drive the analtye(s) through the separation column. This EOF is generated due to an electrical double layer at the interface of the solid support with the liquid mobile phase. Most commonly, silica is used as the solid support and develops a negative surface charge under CE/CEC running conditions, presumably as a result of the deprotonation of the silanol groups. The negatively charged substrate attracts cations from the electrolyte in the mobile phase thereby forming the electrical double layer.

In this invention, the positively charged quaternary ammonium moiety contained in the *N*-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride provided a positively charged surface on the monolithic matrix, which, in turn, counteracted on the effects of the residual silanol groups residing both on the monolith and on the inner capillary surface. Under the experimental conditions used, a strong EOF was observed in the reversed direction (from cathode to anode), suggesting that the surface positive charge due to quaternary ammonium functionality in the surface-bonded C_{18}TMS moieties is the EOF-determining factor in the prepared sol-gel monolithic columns.

The CEC analysis of a mixture of PAHs on a sol-gel ODS monolithic column is shown in FIGURE 5. A separation column 50 cm x 50 μm inner diameter (46.1 cm effective length) is used. The separation conditions were as follows:

Injection	-12 kV for 0.03 min
Run	-15 kV, 2.68 μA
Mobile phase	80% acrylonitrile/20% 5mM Tris-HCl, pH 2.34, DMSO used as the EOF marker
Analytes	(1) benzene 4.4053 x 10^{-6} M
	(2) naphthalene 2.7087 x 10^{-6} M
	(3) impurity
	(4) fluorene 1.5433 x 10^{-6} M

(5) phenanthrene 1.5748 x 10^{-6} M

(6) anthracene 9.6850 x 10^{-7} M

(7) fluoranthene 1.1654 x 10^{-6} M

(8) pyrene 1.2283 x 10^{-6} M

(9) benzo[a]pyrene 1.5118 x 10^{-6} M

The monolithic sol-gel column allowed for the use of a mobile phase containing a higher percentage of acetonitrile (up to 80%) and simultaneously rendered sufficient solute—stationary-phase interactions. The separation efficiencies acquired for naphthalene in the mixture of PAH analytes in this analysis were on the order of 145,800 theoretical plates per meter (73,000 plates/column). Because monolithic columns with overall lengths of up to several meters can be easily prepared by the presented sol-gel technology and that the prepared columns can be operated using commercially available CE instrumentation, new possibilities for generating extremely high efficiencies per column in CEC separations are created.

Van Deemer plots, as depicted in FIGURE 6, were constructed through variations in the operating voltages, thereby altering the mobile-phase flow rate through the column and measuring the achieved plate heights corresponding to each operating voltage. The conditions were as follows:

Injection	-12 kV for 3 sec
Run	-3 to –19 kV
Mobile phase	75% acetonitrile/25% 5 mM Tris-HCl, pH 2.34, DMSO used as the EOF marker
Test solutes	(a) naphthalene
	(b) anthracene

For the used test solutes, the Van Deemer plots reveal minimal increases in plate heights as the mobile-phase flow rates are enhanced. The relatively flat right-hand portion of the H vs **u** curves indicate an efficient mass-transfer process between the mobile phase and the monolithic ODS separation bed.

As can be seen in FIGURE 6, the optimum linear velocity for the used sol-gel monolithic ODS column was 0.75 mm/s, which corresponds to applied field strength of

-240 V/cm (-12 kV) in the sol-gel monolithic columns. This shows a new possibility for use of longer sol-gel columns that produce higher overall column efficiencies without exceeding the upper voltage limits of commercially available CE instruments. Furthermore, the use of sol-gel technology to prepare these monolithic ODS columns for CEC is further accentuated as increased column lengths can be used because the highly porous structure of the monoliths allows for their rinsing and CEC operation using commercially available CE instrumentation without any additional pressurization capability. There was no need for pressurization of both capillary ends during analysis or for increased pressurization for capillary rinsing prior to analysis. No bubble formation was detected during analysis with the monolithic capillaries when using electric field strengths of up to 300 v/cm. In addition, the highly porous structure of the monolithic capillaries allowed for operation without the need for modification to the commercial CE instrument.

A test mixture of benzene derivatives was also used to further evaluate the separation performance of the sol-gel ODS monolithic columns using a mobile phase containing 75% acetonitrile and 25% aqueous 5 mM Tris-HCl at pH of 2.34. Column efficiencies on the order of 163,200 plates/m (81,600 plates/column) were obtained in these analyses as shown in FIGURE 7.

FIGURE 8 illustrates an analogous separation of a mixture of aldehydes and ketones obtained on a sol-gel monolithic ODS column. As in the case with the benzene derivatives, this probe mixture contained more closely related analytes. Column efficiencies on the order of 174,600 plates/m (87,300 plates/column) were obtained in these analyses. The conditions here were:

Separation column	50 cm x 50 μm (inner diameter)
	(46.1 cm of effective length)
Injection	-12 kV for 0.03 min
	-25 kV 0.5 μA run
Mobile phase	70% acrylonitrile/30% Tris-HCl, pH 2.34
Analytes	(a) benzaldehyde, 1.180 x 10^{-3} M
	(b) *o*-tolualdehyde, 1.9655 x 10^{-3} M
	(c) butyrophenone, 3.2680 x 10^{-4} M

(d) valerophenone, 1.5263×10^{-4} M

(e) hexaphenone, 3.0618×10^{-4} M

(f) heptaphenone, 2.986×10^{-4} M.

Repeatability studies were performed using various analyte mixtures. These experiments were essential to evaluate the consistency in solute retention on the sol-gel monolithic ODS columns. The following table presents the CEC characteristics of sol-gel monolithic columns and experimental data on retention time repeatability for a test mixture of seven aromatic aldehydes and ketones.

Analyte	Separation Efficiency, N (plates/column)	t_R (min)	Retention factor, k	Separation Factor, α	s	R.S.D. (n=5)
Benzaldehyde	89 778	9.144	0.050		0.027	0.295%
Tolualdehyde	91 039	9.526	0.094	0.382	0.029	0.302%
Butyrophenone	83 867	10.048	0.154	0.522	0.025	0.248%
Valerophenone	79 353	10.678	0.227	0.630	0.016	0.154%
Hexaphenone	86 027	11.550	0.327	0.872	0.022	0.194%
Heptaphenone	89 687	12.788	0.469	1.238	0.031	0.244%

As depicted in this table, consistent repeatability values are exemplified by the low RSD (0.15-0.30%) values for solute retention times in a series of five consecutive runs.

An additional embodiment of the invention is shown in FIGURE 9. This embodiment provides an optical window that allows for the use of a detection device to monitor the eluted analytes after passing through the separation bed. Such an optical window allows for detection or further analysis of samples.

More specifically, the optical window is formed in an area or segment containing gas **10** of the capillary tube, as generally shown at **12**, adjacent the sol-gel bed **14**. Normally, to provide structural integrity and strength to the tube, an outer coating **16** is provided around the entire outer surface of the tube **12**. This outer coating **16** can be in the form of a polymer, metal or other coating as known in the art. In accordance with this embodiment of the present invention, this outer coating **16** is removed from the tube

adjacent the sol-gel bed **14**, as shown by **18**, defining an optical window **30** in the tube in the area of the gas containing area **10**.

Generally, the capillary tube **12** itself is optically transparent. However, the outer protective coating **16** interferes with the optical properties thereof. In order to provide the optical window **30**, one end of the tube is sealed, as in **20**. Under pressure, the sol-gel solution is introduced, thereby compressing the gas within the capillary tube inner gas containing area **10**. This forces the formation of a compressed gas space **24**, separating an end portion of the bed **14** from the sealed end portion **20**. A portion of the outer coating is removed, by burning or by other means known in the art, in the area of the gas containing area **10**, thus forming the optical window **30**, in the capillary tube. This window may be of any desired size known to those of ordinary skill in the art.

In this embodiment, the separation bed may also be thermally and/or solvent treated as described earlier, and any further necessary pretreatment steps prior to use of the column is considered here as being within the scope of the invention.

A preferred method for forming this window involves the following steps:

(a) selection of an externally coated optically transparent capillary tube, such as one containing a fused-silica inner surface, the coating being any as known to those of ordinary skill in the art, such as polymer, metal or other removable coatings;

(b) sealing of the distal capillary end;

(c) filling the sealed capillary with sol-gel solution, the pressure of the compressed gas in the sealed portion forming a gas pocket in the distal end thereof;

(d) allowing the sol-gel solution inside the capillary to transform into a porous monolithic bed;

(e) sealing the proximal end of the capillary tube to allow thermal conditioning;

(f) removing the seals at both ends of the filled capillary tube to allow for solvent conditioning; and

(g) removing a portion of the outer coating of the capillary tube at the distal end in the region of the former gas pocket to provide a window of any desired length for optical spectrometric analyses.

The incorporation of the optical window allows for additional analyses to be performed on the sample as it exits the separation bed. The choice of spectrometer is within the scope of ordinary skill in the analytical chemistry art, and any known instrument is considered within the scope of the invention. It is also understood that any form of pretreatment of the column may be used including thermal and solvent or both, but the choice of pretreatments is solely a matter of choice.

It may be appreciated by one skilled in the art that additional embodiments may be contemplated, including alternate reagents and tubes used for the outer matrix for the sol-gel filling.

In the foregoing description, certain terms have been used for brevity, clarity and understanding, but no necessary limitations are to be implied therefrom beyond the requirements of the prior art, because such words are used for description purposes herein and are intended to be broadly construed. Moreover, the embodiments of the apparatus and reagents used herein are by way of example, and the scope of the invention is not limited to those in either construction or chemistry.

Having now described the invention, the preferred embodiments thereof and the advantageous new and useful results obtained thereby, along with reasonable chemical equivalents thereof as obvious to those of ordinary skill in the art, these are now set forth in the appended claims.

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CLAIMS

What is claimed is:

1. A method of preparing a monolithic column by forming a monolithic separation bed from a sol-gel solution in a single step.

2. A method as in claim 1, wherein said single step is further defined as simultaneously providing the separation bed, column deactivator, chemically bonded stationary phase ligand, and surface charge for electrostatic flow.

3. A method as in claim 2, further including the steps of chromatographically interacting analytes with the monolithic stationary phase.

4. A method as in claim 3, wherein said interacting step is further defined as incorporating into the sol-gel solution a sol-gel precursor including an chromatographically active moiety.

5. A method as in claim 4, wherein said interacting step is further defined as incorporating *N*-Octadecylidimethyl[3-(trimethoxysilyl)propyl]ammonium chloride into the sol-gel solution which yields a positively charged surface.

6. A method as in claim 4, wherein the chromatographically active moiety is selected from the group consisting of octadecyl, cyanopropyl, octyl, diol, biphenyl, phenyl, cyclodextrins, and crown ethers.

7. A method as in claim 2, wherein the column deactivator is selected from the group consisting of phenyldimethylsilane, octyldimethylsilane, and octadecyldimethylsilane.

8. The monolithic sol-gel column formed by the process of claim 1.

9. A monolithic sol-gel column comprising a stationary phase formed by a one-step process inside a capillary tube.

10. The column of claim 9, wherein the stationary phase is defined as a unitary structure bonded with the walls of said outer capillary tube.

11. A method for making a sol-gel column comprising:

a) selecting an optically transparent capillary tube having a protective outer coating suitable for forming a monolithic column;

b) sealing one end of said tube;

c) adding sol-gel solution to an open end of the tube under pressure to form a filled column with a gas pocket at the sealed end of the tube;

d) solidifying the sol-gel solution to form a bed; and

e) removing a portion of the protective coating in the area of the former air pocket region adjacent the filled portion of the tube to form an optical window.

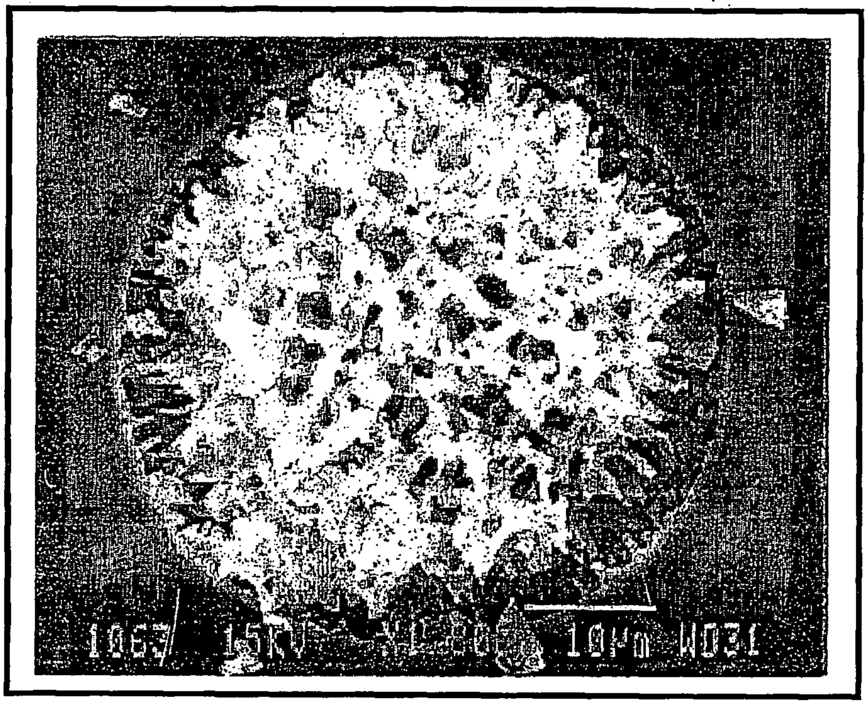
12. The sol-gel column formed by the process of claim 11.

13. A sol-gel column comprising:

a) an optically transparent capillary tube;

b) a bed consisting essentially of a solidified sol-gel solution; and,

c) an optical window means within a portion of said tube for providing for optical analysis of analytes exiting said bed.



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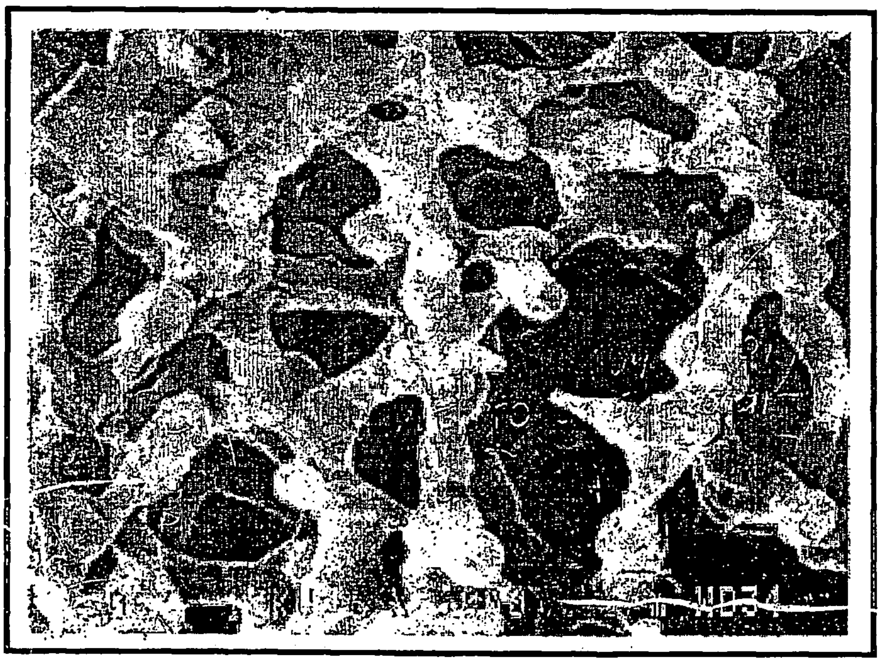


Figure 2

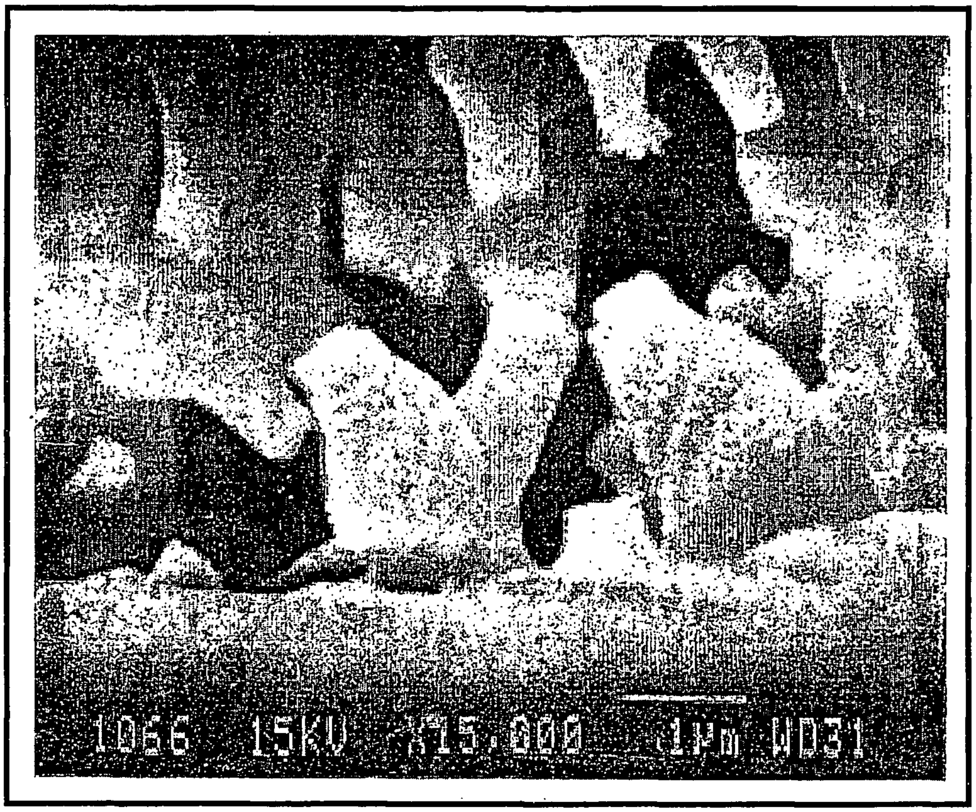
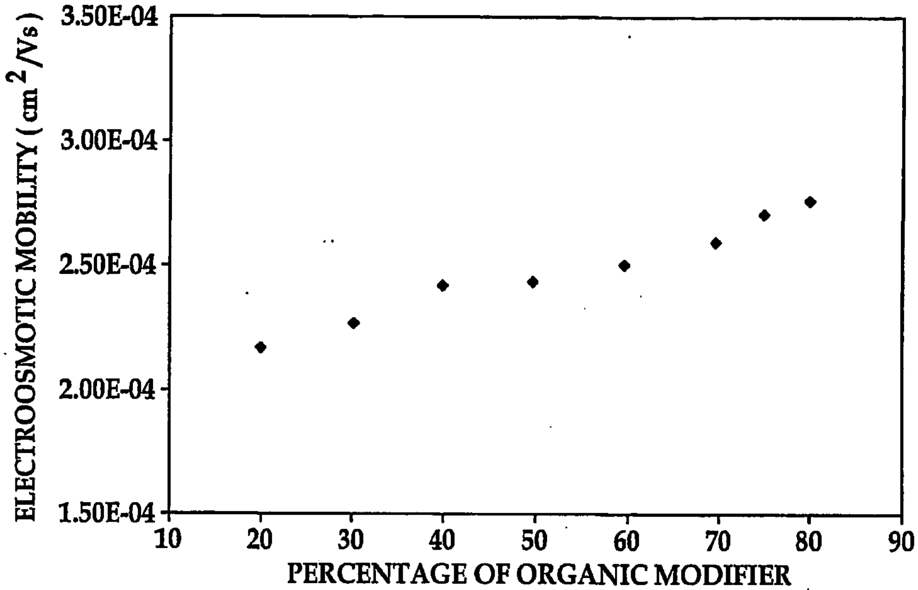


Figure 3



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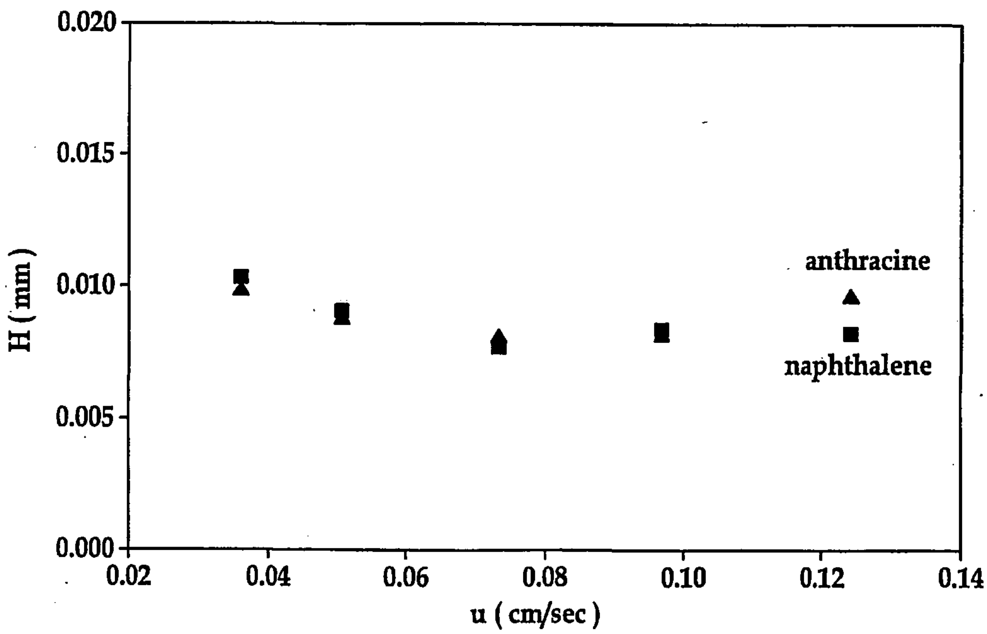

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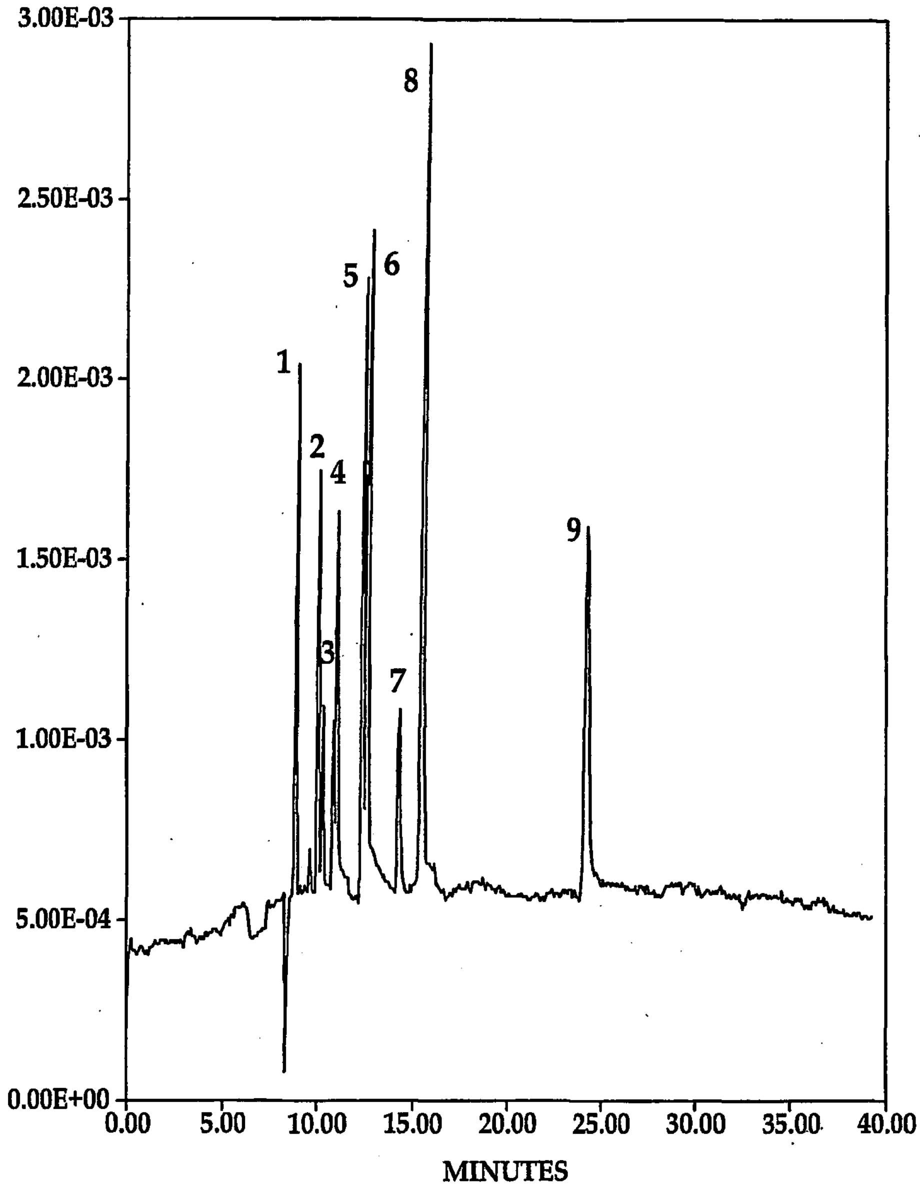
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Figure 5

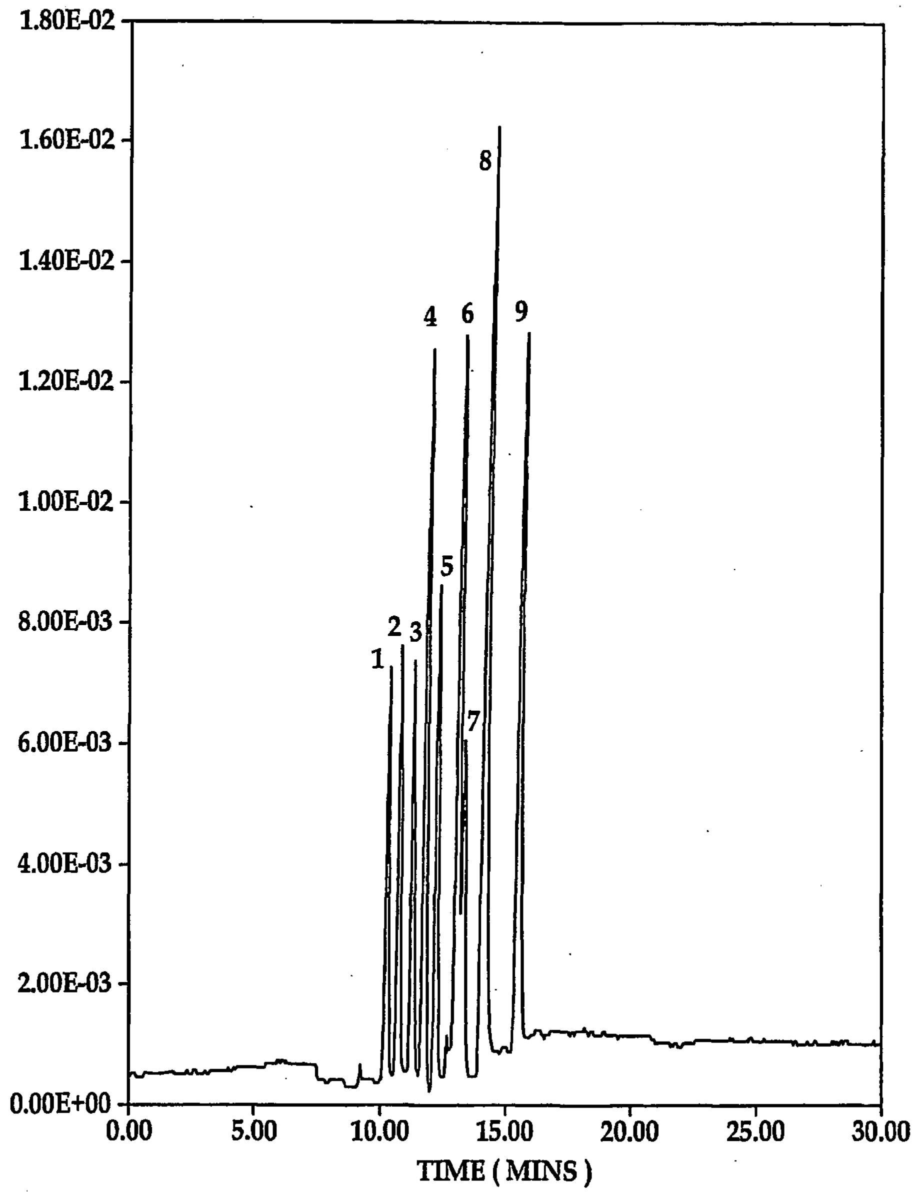
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Figure 7

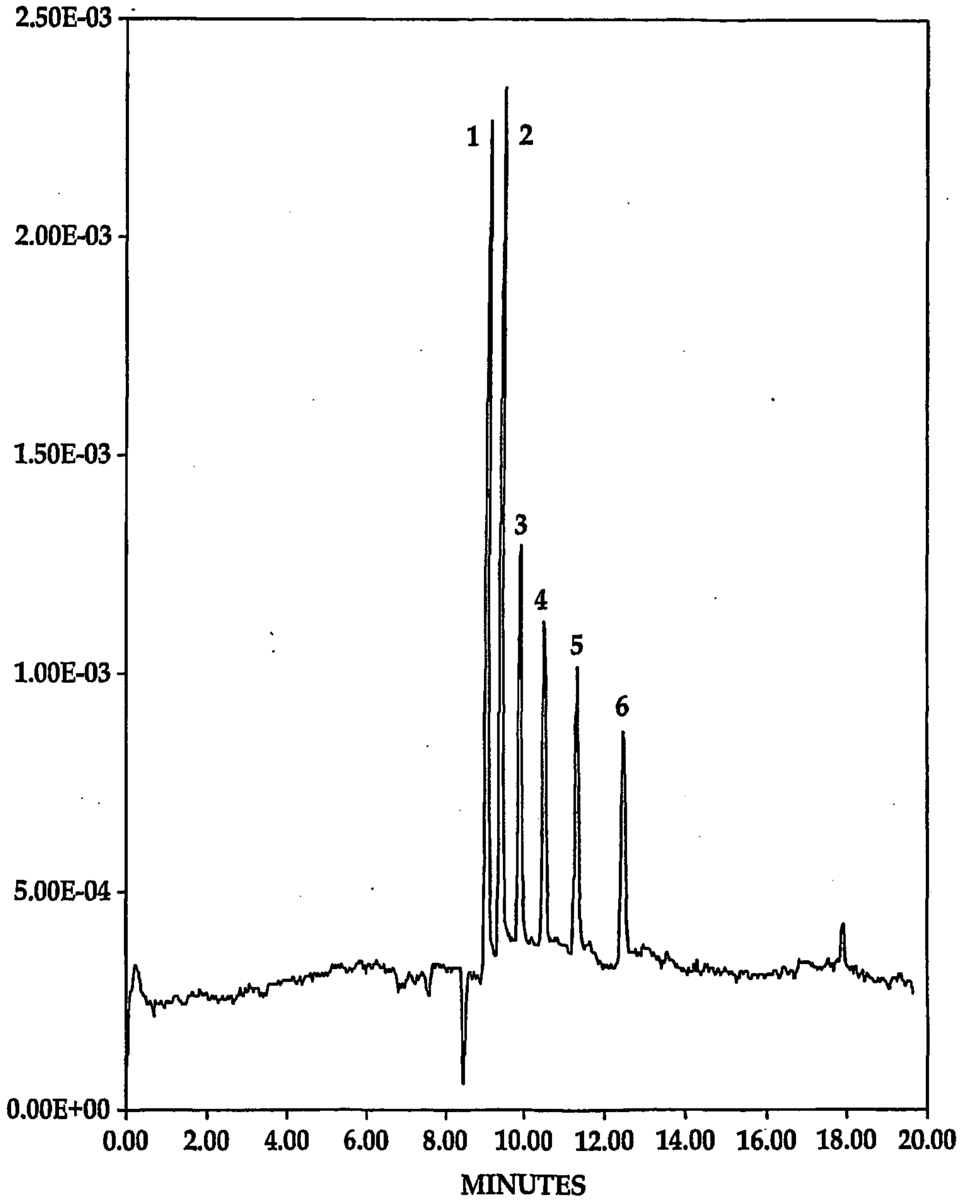
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Figure 8

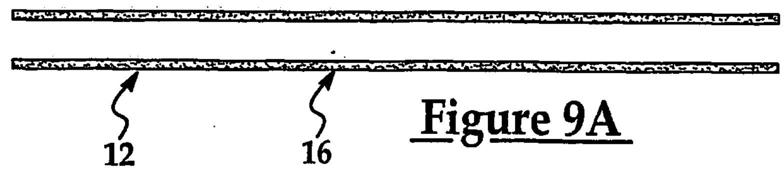

Figure 9A

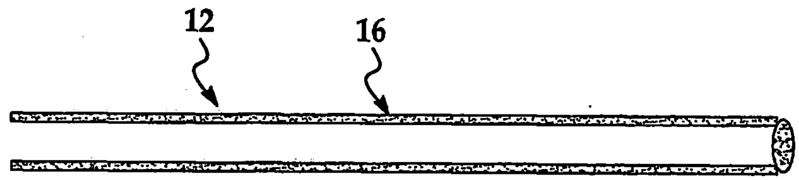

Figure 9B

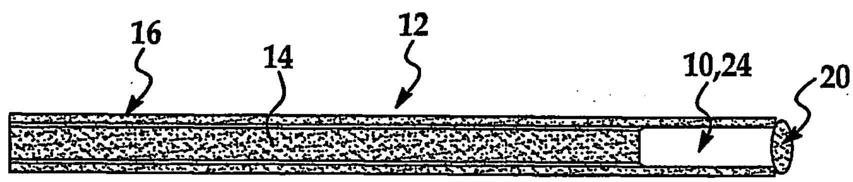

Figure 9C

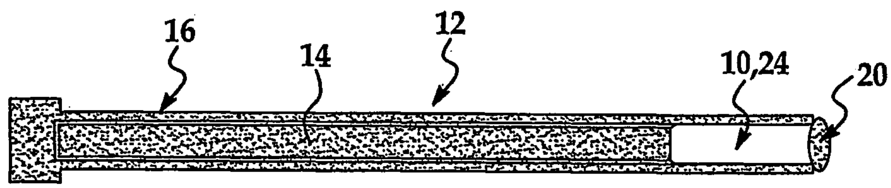

Figure 9D

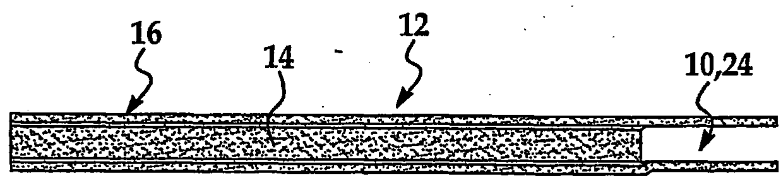

Figure 9E

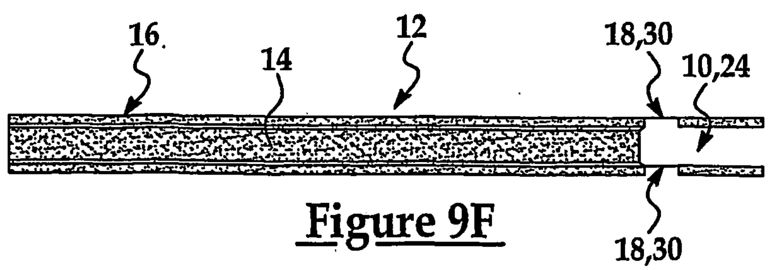

Figure 9F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/04271

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :B01D 15/08
US CL :210/635, 656, 198.2, 502.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/635, 656, 198.2, 502.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,624,875 A (NAKANISHI et al) 29 April 1997, col. 3, lines 12-14.	1-13
Y	US 5,522,994 A (FRECHET et al) 04 June 1996, col. 5, line 65-col. 6, line 1.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 APRIL 2001

Date of mailing of the international search report

25 APR 2001

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